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(54) **Endotoxin-detecting device.**

(57) There is disclosed an endotoxin-detecting device which includes a transparent reaction tube, and an endotoxin reagent sealed in the reaction tube. A specimen liquid is introduced into the reaction tube by either a capillary action or suction, so that the endotoxin reagent is caused to react with the specimen liquid at a predetermined temperature. The concentration of endotoxin contained in the specimen liquid is determined by the gelatinization and coloring of the reaction mixture in the reaction tube.

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ENDOTOXIN-DETECTING DEVICE

This invention relates to an endotoxin-detecting device.

It is believed that endotoxin is lipopolysaccharide present in a cell wall of Gram-negative bacteria. Even a very small amount of endotoxin causes various physiologic activities such as pyrexia. The pyrexia of a living organism caused by endotoxin has been a serious problem in the medical, pharmaceutical and sanitary fields. In recent years, it has been proposed to carry out an endotoxin detection using an endotoxin reagent composed of either amebocyte lysate extracted from blood cells of a horseshoe crab or a mixture of a color producing agent and proenzyme separated from the amebocyte lysate. This method is commonly referred to as "limulus test" in the trade. This method enables endotoxin to be detected much more quickly in comparison with a conventional endotoxin-detecting method in which a small amount of a specimen liquid is applied to a rabbit to see whether the rabbit is subjected to pyrexia.

Various endotoxin-detecting devices for carrying out the above-mentioned limulus test have heretofore been proposed. Such detection devices comprise a reaction container such as a tube holding the above-mentioned endotoxin reagent. For

carrying out the endotoxin detection, a specimen liquid to be examined must be transferred to the reaction container by the use of an endotoxin-free thief tube such as a pipet and a syringe. Therefore, the operation of the conventional endotoxin-detecting devices is rather cumbersome, and in addition there is the risk that the specimen liquid may be contaminated by endotoxin or the like during the transfer of the specimen liquid to the reaction container.

It is therefore an object of this invention to provide an endotoxin-detecting device which obviates the need for a separate thief tube for transferring a specimen liquid to a reaction tube, thereby overcoming the above difficulties of the prior art.

According to the present invention, there is provided an endotoxin-detecting device which comprises a transparent reaction tube, and an endotoxin reagent sealed in the reaction tube.

The reaction tube is sealed at opposite ends thereof by either a heat sealing or closure members such as caps and plugs.

The opposite ends of the reaction tube, when in use, are opened, and one of the open ends is dipped in a specimen liquid so that the specimen liquid is drawn into the reaction tube by the capillary action. The specimen liquid so introduced dissolves the endotoxin reagent to form a mixture

which is heated to a predetermined temperature to react the reagent with the specimen liquid. The reaction mixture in the reaction tube is gelated, becomes turbid or is colored in proportion to the concentration of the endotoxin contained in the specimen liquid. Thus, the presence of endotoxin in the specimen liquid can be easily detected. The reaction tube is of such a diameter that the specimen liquid introduced into the reaction tube is not caused to flow therefrom when it is held horizontally. Preferably, the diameter of the reaction tube should be 0.1 to 5 mm. The reaction tube may be provided at one end thereof with a suction means for positively drawing the specimen liquid into the reaction tube. The reaction tube can be made of any material so long as it has such a transparency that the inside of the reaction tube can be inspected from outside it.

The endotoxin reagent is composed of either amebocyte lysate extracted from blood cells of a horseshoe crab or a mixture of a color producing agent and proenzyme separated from the amebocyte lysate. In view of preservation stability, the endotoxin reagent is preferably in the freeze-dried form. A stabilizing agent may be added to the endotoxin agent.

FIG. 1 is a cross-sectional view of an endotoxin-detecting device provided in accordance with the present invention;

FIG. 2 to 4 are views similar to FIG. 1 but showing modified endotoxin-detecting devices, respectively;

FIG. 5 is a cross-sectional view of another modified endotoxin-detecting device having a membrane container;

FIG. 6 is a cross-sectional view of the endotoxin-detecting device of FIG. 5 taken along the line VI-VI of FIG. 5; and

FIG. 7 is a view similar to FIG. 5 but showing a plurality of reaction tubes sealed in the membrane container.

The invention will now be described with reference to the drawings in which like reference numerals designate corresponding parts in several views.

An endotoxin-detecting device shown in FIG. 1 comprises a straight reaction tube 1 of a uniform diameter having opposite closed ends, and an endotoxin reagent 2 sealed in the reaction tube 1 and disposed intermediate the opposite ends thereof. The tube 1 is made of a transparent material such as glass, polymethyl methacrylate and polystyrene. Preferably, the tube 1 has a small diameter, for example, of 0.1 to 5 mm.

A notch or a line of cut may be formed circumferentially in each of the opposite end portions of the tube 1 to facilitate the removal of these end portions from the tube 1. The tube 1 may have opposite open ends which can be closed by closure members such as plugs and caps, respectively. One or

both of the opposite end portions of the tube 1 may have either smaller or larger diameter than the major portion extending between these opposite end portions. The reaction tube 1 may be formed into a U-shape.

Although the endotoxin reagent 2 is received only in a part of the tube 1, the reagent may be filled in the tube 1 along the entire length thereof.

For determining the concentration of endotoxin contained in a specimen liquid to be examined, the opposite ends of the tube 1 are first removed or cut off so that the tube has opposite open ends. Then, one of these open ends is dipped in a specimen liquid so that the specimen liquid is introduced into the tube 1 by the capillary action. The specimen liquid introduced into the reaction tube 1 dissolves the endotoxin reagent 2 to form a mixture. Then, the tube 1 holding the mixture is placed in a dry warming device to heat the mixture to a predetermined temperature so that the endotoxin reagent 2 is caused to react with the specimen liquid. Then, the tube 1 is tilted to see whether the reaction mixture in the tube 1 is subjected to gelation. At the same time, the reaction tube 1 is observed to determine whether the reaction mixture in the tube 1 is subjected to turbidity or coloring. The degree of gelation and turbidity of the reaction mixture are proportional to the concentration of endotoxin in the specimen liquid. Thus, the concentration of the endotoxin can be easily determined.

As described above, the tube 1 serves as both a thief tube and a reaction tube. Thus, the endotoxin-detecting device according to the present invention obviates the need for a separate endotoxin-free thief tube for taking the specimen liquid. Therefore, with this endotoxin-detecting device, the specimen liquid does not need to be transferred from a thief tube to a reaction tube, so that the detection of endotoxin can be carried out easily. In addition, the risk of contamination of the specimen liquid by endotoxin or the like can be substantially reduced. Further, the reaction tube 1 is of such a small diameter that it holds a relatively small amount of specimen liquid. Therefore, the amount of the reagent for endotoxin in the tube can be small.

FIG. 2 shows a modified endotoxin-detecting device which differs from the endotoxin-detecting device of FIG. 1 in that a transparent tube 1 has a colored portion 3 extending along the length thereof. The colored portion 3 is formed by a color coating applied to a half of the outer circumferential surface of the tube 1 and extending substantially along the entire length thereof. The colored portion has a white color. The colored portion 3 may be provided on one fourths to three fourths of the outer circumferential surface of the tube 1. The colored portion 3 does not necessarily be provided substantially along the entire length thereof, and it may be provided only at the area of the reaction tube 1 where an endotoxin reagent 2 is positioned. Also, the colored portion 3 may be formed by a colored film adhesively bonded to the outer circumferential surface of the reaction

tube 1. Further, the colored coating or the colored film may be applied to the inner circumferential surface of the tube 1. The color of the colored portion 3 may be one other than a white color so long as it is opaque.

A specimen liquid is introduced into the reaction tube 1 in the same manner as described above for the endotoxin-detecting device of FIG. 1. By virtue of the provision of the colored portion 3, the gelation and coloring of the reaction mixture of the reagent 2 and the specimen liquid can be easily observed with naked eyes if they develop.

FIG. 3 shows another modified endotoxin-detecting device which differs from the endotoxin-detecting device of FIG. 1 in that a reaction tube 1 has an open end to which a suction member 4 is attached. The suction member 4 is in the form of a bulb and is made of an elastic material such as rubber and a synthetic resin. The suction member 4 is snugly fitted on the open end of the reaction tube 1 in an air-tight manner and is fixed thereto. The suction member 4 may be formed integrally with the reaction tube 1.

In operation, the closed end of the reaction tube 1 is first removed, and the suction member 4 is squeezed by fingers. Then, the end of the tube 1 remote from the suction member 4 is dipped in a specimen liquid, and the pressure on the suction member 4 is reduced or released so that the specimen liquid is drawn into the reaction tube 1 by suction. Then, the gelation and coloring of the reaction mixture of an endotoxin reagent 2 and the specimen liquid are observed in the same manner as described above for the endotoxin-

detecting device of FIG. 1 to determine the concentration of endotoxin contained in the specimen liquid. By virtue of the provision of the suction member 4, the liquid specimen can be introduced into the tube 1 easily and positively. The suction member 4 may be of any shape so long as it can draw the specimen liquid into the reaction tube 1 by suction. Also, the tube 1 may have a colored portion for facilitating the observation of the reaction mixture in the tube 1, as described above for the endotoxin-detecting device of FIG. 2.

FIG. 4 shows a further modified endotoxin-detecting device which differs from the endotoxin-detecting device of FIG. 1 in that a reaction tube 1 is provided with a graduated scale 5. A notch or a line 6 of cut is formed circumferentially in each of the opposite end portions of the tube 1 to facilitate the removal of the opposite end portions from the tube 1. The graduated scale 5 comprises a pair of lines each formed circumferentially around the reaction tube 1 and disposed intermediate an endotoxin reagent 2 and a respective one of the notches 6. A plurality of scale lines 5 may be provided on the tube 1 between the reagent 2 and a respective one of the notches 6. Also, the scale lines 5 may be replaced by dots. Alternatively, the reaction tube 1 may have regions of a transparent color which replace the scale lines 5. Further, although the reagent 2 is received in the tube 1 at a central portion thereof, the reagent may be disposed at any position between the opposite scale lines 6.

In operation, the opposite ends of the tube 1 are broken off from the reaction tube 1 at the respective notches 3. Then, one of the opened ends of the reaction tube 1 is dipped in a specimen liquid to draw it into the tube 1 up to the scale line 5 by the capillary action. Then, the gelation and turbidity of a reaction mixture of the reagent 2 and the specimen liquid are observed to determine the concentration of endotoxin in the specimen liquid. By virtue of the provision of the graduated scale 5, a constant amount of the specimen liquid is always introduced into the reaction tube 1, so that the concentration of endotoxin in the specimen liquid can be accurately determined with reproducible results.

The reaction tube 1 may have a colored portion for facilitating the observation of the reaction mixture in the tube 1, as described above for the endotoxin-detecting device of FIG. 2. Also, the reaction tube 1 may be provided at one end with a suction member for positively drawing the specimen liquid into the tube 1, as described above for the endotoxin-detecting device of FIG. 3.

FIG. 5 shows a still further modified endotoxin-detecting device which differs from the endotoxin-detecting device of FIG. 1 in that a reaction tube 1 has opposite open ends and in that the reaction tube 1 is hermetically sealed in a membrane container 7.

The membrane container 7 comprises a pair of identical rectangular films 7a and 7b between which the tube 1 is sandwiched, the films 7a and 7b being hermetically sealed or

bonded together along their entire peripheral margins by either heat-sealing or a suitable adhesive, thereby providing a peripheral sealed portion 8. A triangular notch 9 is formed in the peripheral sealed portion 8 for facilitating the tearing of the membrane container 7 when in use. The notch 9 may be replaced by a slit. Each of the films 7a and 7b comprises a film impermeable to moisture, such as a laminated aluminum film incorporating a polymer, a polyvinylidene film, a polyolefin film and an ethylene vinyl chloride copolymer film.

The membrane container 7 may be made of a single square film folded in two, with three open sides of the folded film being sealed. Alternatively, the membrane container 7 may be made of a film tube with opposite open ends being sealed, in which case a fused portion is formed on the membrane container in which fused portion the notch 9 is provided. Also, the membrane container 7 may be vacuum sealed. A desiccant may be contained in the membrane container 7 together with the reaction tube 1. A plurality of reaction tubes 1 may be sealed in the membrane container 7 as shown in FIG. 7.

The provision of the membrane container 7 obviates the need for directly closing the opposite open ends of the reaction tube 1 by either heat-sealing or closure members such as caps and plugs after an endotoxin reagent 2 is filled in the tube 1. Therefore, the risk of contamination of the reaction tube 1 by endotoxin or the like when sealing the tube 1 can be substantially reduced. In addition, the inner

and outer surfaces of the membrane container 7 can be preserved in an endotoxin-free condition. And, in the case where a plurality of reaction tubes 1 are sealed, much time and labor can be saved.

The reaction tube 1 may have a colored portion for facilitating the observation of the reaction mixture in the tube 1, as described above for the endotoxin-detecting device of FIG. 2. Also, the reaction tube 1 may be provided at one end with a suction member for positively drawing the specimen liquid into the tube 1, as described above for the endotoxin-detecting device of FIG. 3. Further, the tube 1 may be provided with a graduated scale for introducing a constant amount of the specimen liquid into the tube 1, as described above for the endotoxin-detecting device of FIG. 4.

Claims:

1. An endotoxin-detecting device comprising a transparent reaction tube, and an endotoxin reagent received in said tube.

2. An endotoxin-detecting device according to claim 1, in which said endotoxin reagent is hermetically sealed in said tube.

3. An endotoxin-detecting device according to claim 1, in which said reaction tube has a diameter of about 0.1 to 5 mm.

4. An endotoxin-detecting device according to claim 1, in which said reaction tube has a peripheral wall at least part of which is colored to provide a colored portion, said colored portion being interrupted in the direction of the periphery of said reaction tube.

5. An endotoxin-detecting device according to claim 1, in which said reaction tube has a suction means provided at one end thereof for drawing a specimen liquid into said reaction tube.

6. An endotoxin-detecting device according to claim 1, in which said reaction tube has a graduated scale.

7. An endotoxin-detecting device according to claim 1, further comprising a membrane container in which said reaction tube is hermetically sealed.

8. An endotoxin-detecting device according to claim 7, in which a plurality of reaction tubes are hermetically sealed in said membrane container.

9. An endotoxin-detecting device according to claim 7, in which said membrane container has a notch formed therein at a central portion thereof for facilitating the tearing of said membrane container.

10. An endotoxin-detecting device according to claim 1, in which said endotoxin reagent is in the freeze-dried form.

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FIG. 1

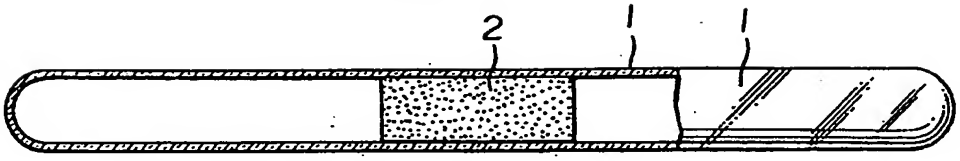

FIG. 2

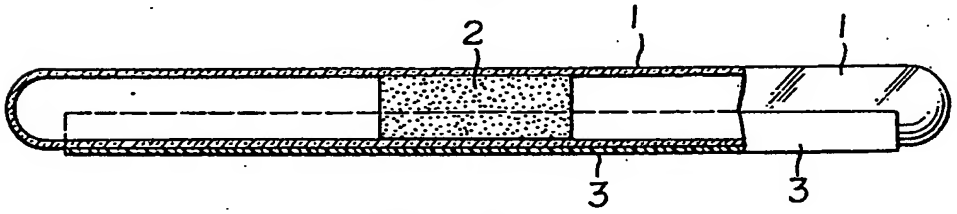

FIG. 3

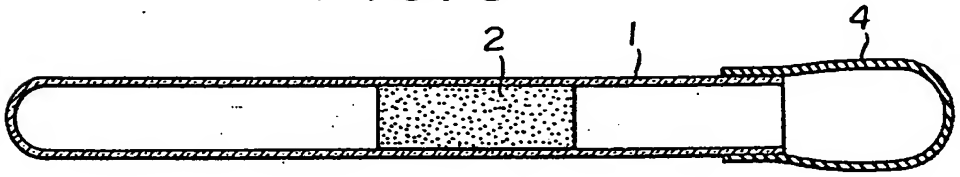

FIG. 4

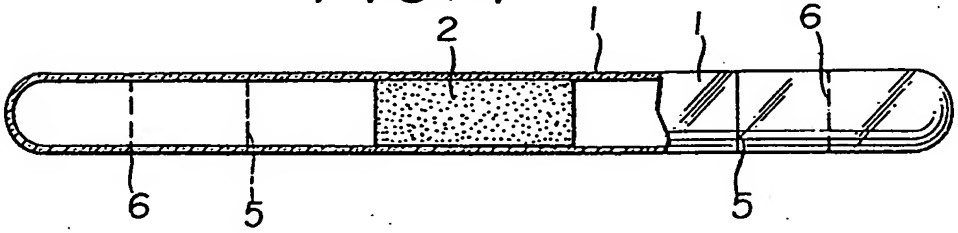

FIG.5

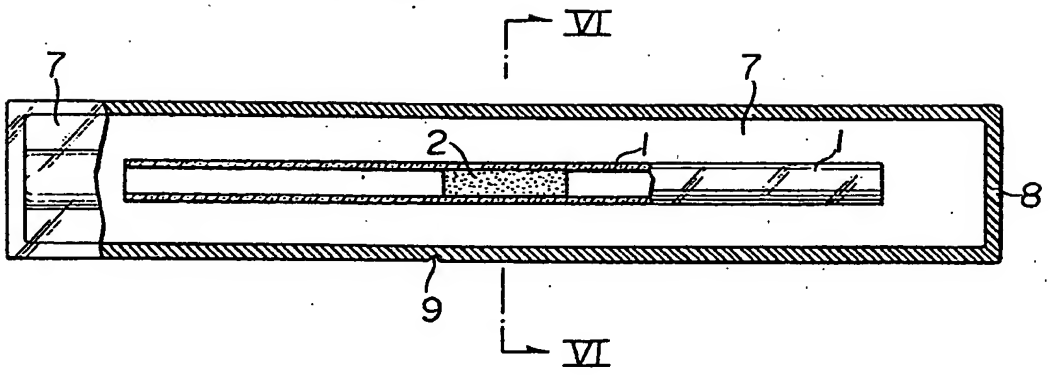

FIG.6

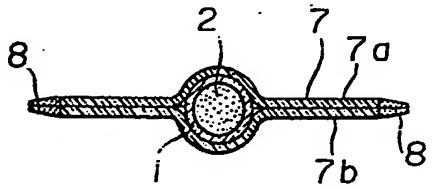

FIG.7